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PPARγ transcriptionally regulates the expression of insulin-degrading enzyme in primary neurons

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ABSTRACT

Insulin-degrading enzyme (IDE) is a protease that has been demonstrated to play a key role in degrading both Aβ and insulin and deficient in IDE function is associated with Alzheimer's disease (AD) and type 2 diabetes mellitus (DM2) pathology. However, little is known about the cellular and molecular regulation of IDE expression. Here we show IDE levels are markedly decreased in DM2 patients and positively correlated with the peroxisome proliferator-activated receptor γ (PPARγ) levels. Further studies show that PPARγ plays an important role in regulating IDE expression in rat primary neurons through binding to a functional peroxisome proliferator-response element (PPRE) in IDE promoter and promoting IDE gene transcription. Finally, we demonstrate that PPARγ participates in the insulin-induced IDE expression in neurons. These results suggest that PPARγ transcriptionally induces IDE expression which provides a novel mechanism for the use of PPARγ agonists in both DM2 and AD therapies.

Introduction

The initial pathological cause of Alzheimer's disease (AD) has been ascribed to the accumulation of amyloid-β (Aβ) [1]. The level of Aβ is determined by the balance between its generation and turnover [2]. Aβ is degraded by endopeptidases, among which insulin-degrading enzyme (IDE) is a major one. IDE mediates much of the degradation of soluble monomeric Aβ [3]. Genetic studies have revealed a significant linkage of IDE gene mutations to AD risks and plasma Aβ levels [4,5]. The study of IDE knocked out mice revealed that the levels of Aβ in the brain are significantly increased [6]. Overexpression of IDE in neurons markedly reduces brain Aβ levels and rescues the premature lethality in APP transgenic mice [7]. Recent studies indicated that IDE gene also contributes to the pathogenesis of type 2 diabetes mellitus (DM2) [8,9]. IDE exists in all insulin-sensitive cells, which share feature of insulin uptake and degradation. Abnormalities in insulin degradation are integral to DM2 [10]. Intracellular insulin is degraded by IDE in endosome [11], which is required for generating insulin effects [12]. In conclusion, abnormality of IDE function could contribute to pathogenesis of both AD and DM2. However, the transcriptional regulation of IDE is not well understood.

A class of insulin sensitizing drugs for the treatment of DM2, Thiazolidinediones (TZDs) have been advanced as a new approach to treat AD [13]. TZDs are agonists of peroxisome proliferator-activated-receptor γ (PPARγ), one of ligand-inducible transcription factors—the peroxisome proliferator-activated receptors (PPARs) which regulate gene expression through multiple mechanisms and function as obligate heterodimers with retinoid-X receptors (RXRs). Upon ligand activation, the PPAR/RXR heterodimer recruits coactivators and binds to sequence-specific peroxisome proliferator-response elements (PPREs) present in the promoter of its target genes [14]. Many studies have demonstrated that PPARγ has an important role in glucose homeostasis and insulin sensitization [15,16]. On the other hand, genetic studies suggested that PPARγ 2 Pro 12 Ala polymorphism is associated with higher risk of AD [17,18]. It is recently reported that PPARγ influences the homeostasis of Aβ in brain [13]. However, the molecular mechanism underlying the effect of PPARγ on brain Aβ levels is unclear.

In the present study, we observed that the levels of IDE are significantly decreased in DM2 patients and positively correlated with PPARγ. We also investigated the transcriptional regulation of IDE by PPARγ.

Materials and methods

Handling of human sample. 30 patients with type 2 diabetes mellitus were collected for 5 years. All patients aged from 38 to 69 years old, and male and female patients were balanced. Thirty healthy control volunteers, matched for age, sex and lifestyle, were recruited from the local community. All participants underwent physical examination and completed questionnaire on medical history, family diabetic history, and smoking habit. A resting 12-lead

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electrocardiogram was performed, and angina was excluded for each patient according to the criteria of the World Health Organization Rose questionnaire. The local Ethical Committee had approved the study protocol.

Primary neuronal culture. Hippocampi and cerebellum were dissected from the brains of embryonic day 18 (E18) Sprague Dawley rat fetuses, treated with 0.05% trypsin in HBSS for 5 min at 37 °C, and dissociated by repeated passage. Nerve cells were grown in Neurobasal medium (NBM; Invitrogen, Carlsbad, CA, USA) supplemented with B27, 5 U/ml penicillin, 5 μg/ml streptomycin, 0.5 mM glutamine, and 25 μM glutamate at 37 °C in 10% CO_2. The culture media were exchanged with glutamate-free NBM 3 days after the start of cell culture. Cultures grown in serum-free NBM yielded 99.5% neurons and 0.5% glia after 7–10 days of culture and were then treated with PPARγ agonists or other pharmacological agents in B27-free NBM.

Western immunoblotting. Protein concentrations were determined by use of the Amersham Pharmacia Biotech electrophoresis machine. First, samples (50 μg of total protein/lane) were separated by electrophoresis through SDS–PAGE gels and transferred to PVDF membranes (Millipore, Bedford, USA). After transfer, the membranes were blocked for 1.5 h in TTBS containing 5% nonfat milk. The membranes were then incubated with primary antibodies specific for IDE (diluted 1:4000, Abcam, USA) PPARγ (diluted 1:1000, Abcam, USA) or β-actin (diluted 1:500, Santa Cruz Biotechnology, USA). After they were rinsed in TTBS, the membranes were incubated with horseradish peroxidase-conjugated anti-mouse IgG (1:10,000) or anti-rabbit IgG (1:10,000) in TTBS. The membranes were developed by Kodak medical X-ray processors (Kodak, Tokyo, Japan).

Immunocytochemical staining. Neurons were cultured on poly-D-lysine-coated slides. The slides were fixed with 95% methanol (−20 °C, 5 min) and blocked for 30 min. They were then incubated with primary antibodies specific for IDE (diluted 1:500, Calbiochem, USA) for 60 min, followed by an anti-rabbit IgG conjugated with FITC (diluted 1:250; Vector Laboratories, USA) for 30 min. Finally, the slides were coverslipped with DAPI-containing mounting medium (Vector Laboratories, CA, USA). They were examined using an inverted fluorescence microscope (Leica DMLB and MPS60, Leica Microsystems Wetzlar GmbH, Germany).

Real-time PCR. Cells were collected and total mRNA was isolated using Trizol (Invitrogen, Carlsbad, CA, USA). Probes for rat IDE TaqMan Real-Time PCR were purchased from Applied Biosystems (Foster City, CA, USA, assay on Demand #Mm00473077_m1). The

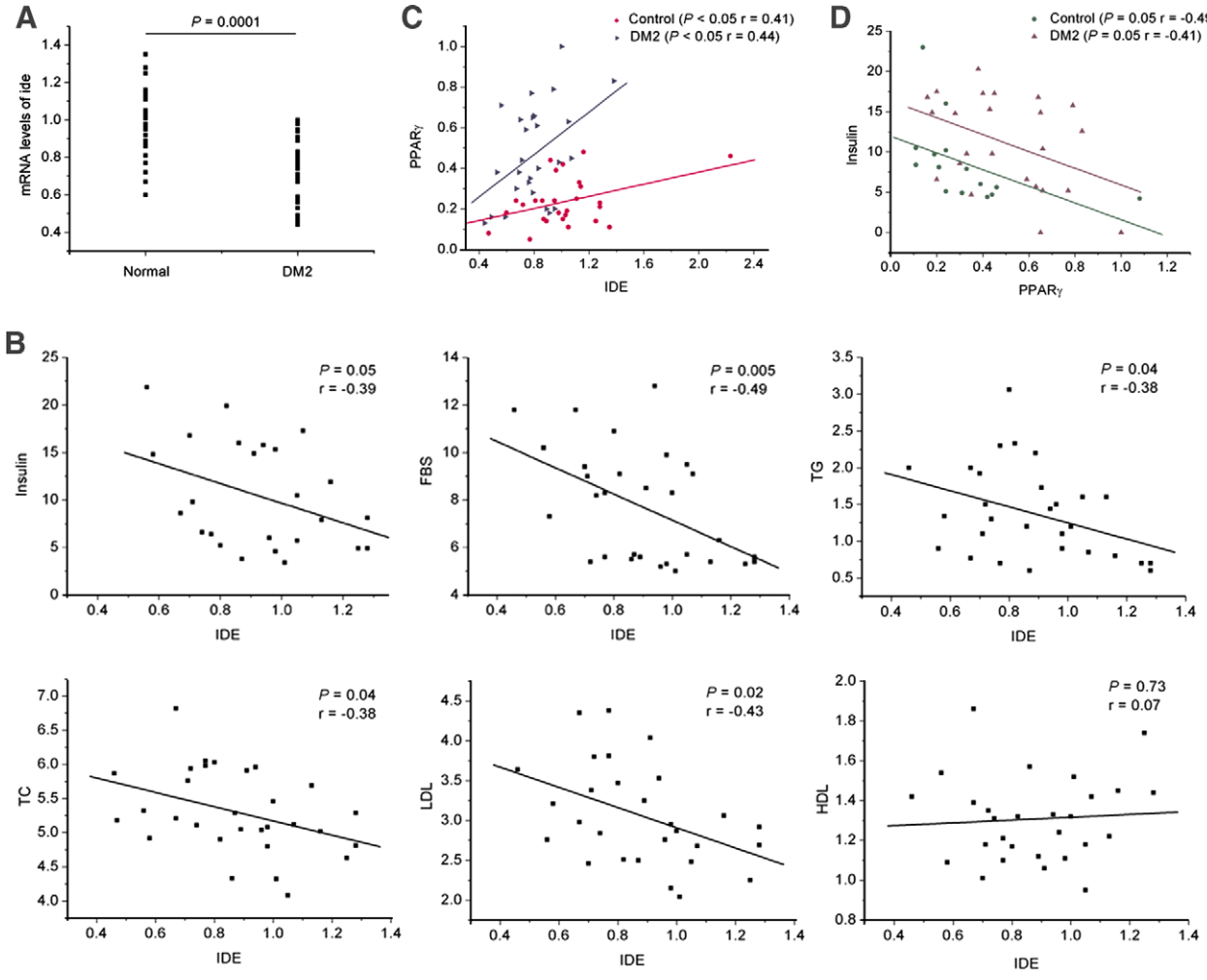

Fig. 1. Levels of IDE are decreased in DM2 patients and associated with PPARγ. (A) IDE mRNA levels were compared between DM2 patients and normal control by Real-time PCR. $P = 0.0001$, $n = 29$. (B) IDE levels were significantly inversely correlated with plasma insulin ($r = -0.39$, $P = 0.05$, $n = 25$), fasting blood glucose (FBS; $r = -0.49$, $P = 0.005$, $n = 30$), triglyceride (TG; $r = -0.38$, $P = 0.04$, $n = 29$), total cholesterol (TC; $r = -0.38$, $P = 0.04$, $n = 30$), low-density lipoprotein (LDL; $r = -0.43$, $P = 0.02$, $n = 28$), but not high-density lipoprotein (HDL; $r = 0.07$, $P = 0.73$, $n = 28$). (C) IDE and PPARγ mRNA levels were significantly correlated in DM2 patients ($r = 0.44$, $P < 0.05$, $n = 26$) and control ($r = 0.41$, $P = 0.05$, $n = 27$). (D) PPARγ mRNA levels were inversely correlated with plasma insulin in DM2 patients ($r = -0.41$, $P = 0.05$, $n = 24$) and control ($r = -0.49$, $P = 0.05$, $n = 15$).